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Cytological Studies on F_1 and F_2 Generations of the Hybrid between *Triticum durum* × *T. aestivum* with Rye B Chromosomes

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Summary

Cytological investigation on F_1 and F_2 generations of the hybrid between *Triticum durum* and *T. aestivum* with many B chromosomes of rye was carried out. The chromosomes of the D genome mostly remained as univalent in both the generations although occasional heterovalents were observed with the chromosomes of the A and B genomes. In both the pentaploid F_1 and hypo-pentaploid F_2 plants, rye B chromosomes have no effect on pairing of wheat chromosomes. The number of B chromosomes was positively correlated with the frequency of laggards at anaphase-I in both the generations due to a less pairing affinity between the B chromosomes. The univalent A chromosomes rapidly lost from the hybrids while rye B chromosomes were less eliminated.

Key words : wheat, pentaploid F_1 and F_2 hybrids, B chromosomes of rye, cytology, chromosome pairing.

Introduction

The common wheat, *Triticum aestivum,* is an allo-hexaploid consisting of three genomes A, B and D. The genomes were derived from three different diploid ancestors. In the hybrids between these diploid ancestors, considerable chromosome pairing occurs regularly, indicating that the homoeologous chromosomes of the diploids are very closely related[1]. But in the hexaploid wheat no association takes place between the homoeologous chromosomes due to a pairing suppresser activity of the $5B^L$ chromosome [2, 3, 4]. The F_1 hybrids between *T. aestivum* and either of the two diploid species of *Aegilops speltoides* and *A. mutica* reveal high levels of homoeologous chromosome pairing in the presence of chromosome 5B[5, 6]. However, the high levels of homoeologous chromosome pairing in the hybrids between *T. aestivum* × *Ae. mutica*[7, 8] and *T. aestivum* × *Ae. speltoides*[9, 10] were drastically reduced in the presence of B chromosomes of the above two *Aegilops* species in the presence or absence of chromosome 5B. One further factor that adds complexity to the pairing control system in wheat arises from an increased dose of the $5B^L$, leading to the progressive increase in the degree of asynapsis of homologous and homoeologous chromosomes[1, 11, 12]. In the present investigation pentaploid F_1 hybrids between *T. durum* × *T. aestivum* and their hypo-pentaploid F_2 generation were cytologically analyzed for $5B^L$ activity on the homoeologous pairing in the absence or presence of an increased

dose of rye B chromosomes.

Materials and Methods

The materials comprised of the F_1 and F_2 generations of the hybrids with 1 to 5 B chromosome of rye derived from the cross between *T. durum* (AABB) × *T. aestivum* (AABBDD+Bs) carrying rye B chromosomes. For somatic chromosome analysis, root tips were collected from 3 days old seedlings germinated on moist filter paper in petri dish. They were then pretreated with ice water at 0°C for 24 hours and fixed in acetic-alcohol (1:3). At flowering stage young spikes were collected and fixed in ethanol-chloroform -acetic acid (6:3:2) for cytological observation of chromosome pairing in the pollen mother cells. Both the root tip and pollen mother cells were stained and squashed in Snow's carmine[13] for recording the data.

Results

A. Meiosis in F_1 Plants

Most of the plants in the F_1 generation of the hybrids possess anexpected pentaploid chromosome number of 35 As along with a variable number of B chromosomes which ranged from 1 to 5 (Fig. 1). Both the standard type (stB) as well as derivatives of stB chromosome were recorded. Pentaploid plants with no B chromosomes served as control. The average univalent per cell at meiotic metaphase I in plant series with 1 to 5 B chromosomes showed a little increase with an increase in the number of B chromosomes (Table 1, Fig. 2 A-D). The average A univalent ranged from 6.98 to 8.79 per cell and the correlation coefficient with the number of B chromosomes was nonsignificant. Similarly, the correlation between the average bivalent of the A chromosomes and the number of Bs was also nonsignificant. The average trivalent per cell was increased slightly but showed no significant correlation. A very little number of tetravalent were observed in plants with B chromosomes. The modal configuration of the A chromosomes showed in most cases with 7 I +14II. The average univalent of the B chromosomes in the above plant series ranged from 0.98 to 2.34 per cell. The average bivalent per cell was fewer than expected, while trivalent was extremely lower.

The frequencies of chromosomes involved in F_1 plants were estimated in percentage (Table 2). The percentage in each configuration corresponds to the number of chromosomes involved in uni-, bi- or multi-valents in terms of the total chromosomes and calculated separately for A and B chromosomes. In both the control plants and the plants with 1 to 5 B chromosomes, the percentage of A chromosomes remained univalent and was almost similar in number. The percentage of bivalent formation was slightly decreased in plants with B chromosomes compared to the control but no consistency was observed with the increased number of B chromosomes. Trivalent formation also increased slightly due to B chromosomes, but did not correspond to the number of B chromosomes in the plants. About 50 % of the B chromosomes remained unpaired and most of the remaining Bs paired

Table 1 chromosome association at M-I in the F_1 hybrids with B chromosomes.

Plants	A chromosome association					B chromosome association				Number of cells
	Average				Mode	Average			Mode	
	I	II	III	IV		I	II	III		
35+0B	7.86 (5-13)	13.45 (11-15)	0.08 (0-1)	0.0 —	7 I +14 II	—	—	—	—	253
35+1B	7.10 (3-12)	12.72 (10-14)	0.79 (0-3)	0.02 (0-1)	7 I +14 II	1.0 —	—	—	1 I	173
35+2B	8.09 (5-13)	13.39 (10-15)	0.04 (0-1)	0.0 —	7 I +14 II	1.06 (0-2)	0.47 (0-1)	—	1 II	149
34+2B	8.69 (6-14)	12.65 (10-14)	0.0 —	0.0 —	8 I +13 II	0.98 (0-2)	0.51 (0-1)	—	1 II	149
35+3B	6.98 (5-13)	12.82 (9-14)	0.76 (0-3)	0.02 (0-1)	9 I +13 II	1.44 (0-3)	0.75 (0-1)	0.02 (0-1)	1 I +1 II	219
35+4B	7.45 (5-13)	13.18 (11-15)	0.37 (0-2)	0.02 (0-1)	7 I +14 II	1.86 (0-4)	1.07 (0-2)	0.0 —	2 I +1 II	123
35+5B	8.79 (4-13)	12.35 (10-14)	0.50 (0-3)	0.0 —	11 I +12 II	2.34 (1-5)	1.31 (0-2)	0.01 (0-1)	3 I 1 II	228
Correlation[1]	0.13	-0.43	0.37	0.19						

* : Figures in parentheses indicate the range.
[1] : Degree of freedom, 5.

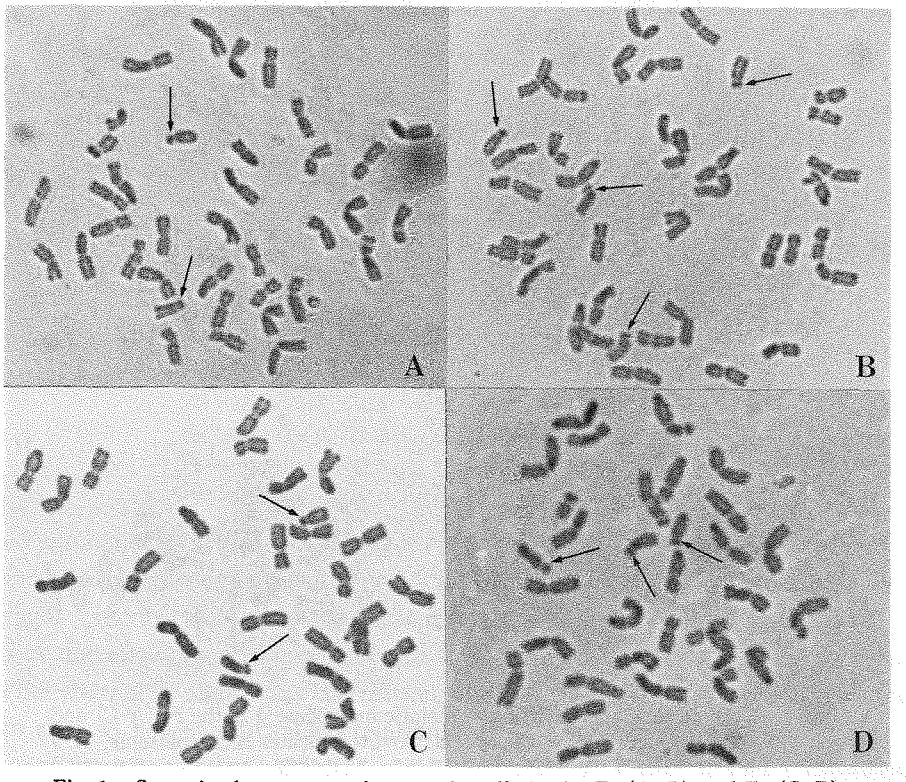

Fig. 1 Somatic chromosomes in root-tip cells in the F_1 (A, B) and F_2 (C, D) generations. Arrow indicates B chromosome. A, 2n=35A+2stB. B, 35A+4stB. C, 29A+2stB. D, 30A+3stB.

into bivalent in the plant series with 1 to 5 B chromosomes. Multivalents were very meager.

Unlike in M-I pairing, the average number of laggards per cell was remarkably higher in the plants with B chromosomes as compared to the control (Table 3, Fig. 3 A-B). The average frequency of lagging chromosomes increased steadily as the number of Bs increased in the plants and showed a significant positive correlation with the number of B chromosomes. The range and the modal number of laggards were also constantly increased due to a higher number of B chromosomes in the plants. In contrast to laggards at A-I, the average frequencies of micronuclei at dyad and at tetrad showed no considerable difference between control plants and the plants with B chromosomes and the correlation coefficients were nonsignificant.

B. Meiosis in F_2 plants

In F_2 generation both hypo- and hyper-pentaploid types of plants regarding the number of A chromosomes were recorded. In the present study hypo-pentaploid plants with A chromosome number closer to the tetraploid mother plants (Fig. 1 C-D) were

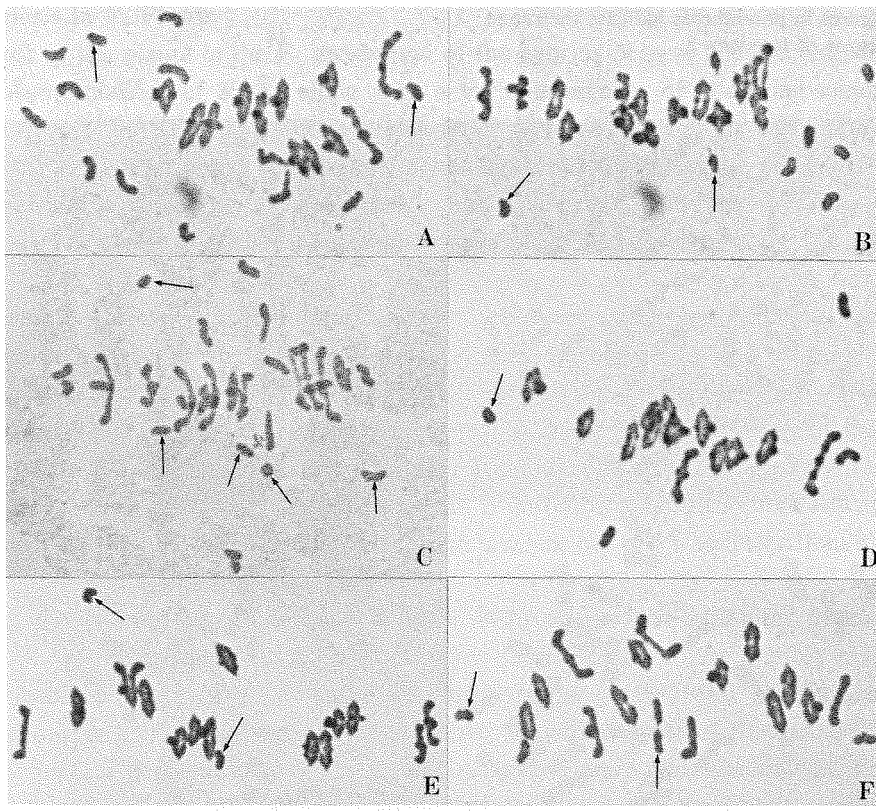

Fig. 2 Chromosome configuration at meiotic metaphase-1 in the F_1 (A-C) and F_2 (D-F) generations of the hybrid. Arrow indicates B chromosome. A, 9 I +13II +2stB I (35+2B plant). B, 5 I +12II +2III +1stB I+1stBII (35+3B plant). C, 11 I +12II +3stB I +2liB I (35+5B plant). D, 3 I+13II +1stB I (29+1B plant). E, 13II +1III+2stB I (29+2B plant). F, 2 I+14II +1stB I+1stBII (30+3B plant).

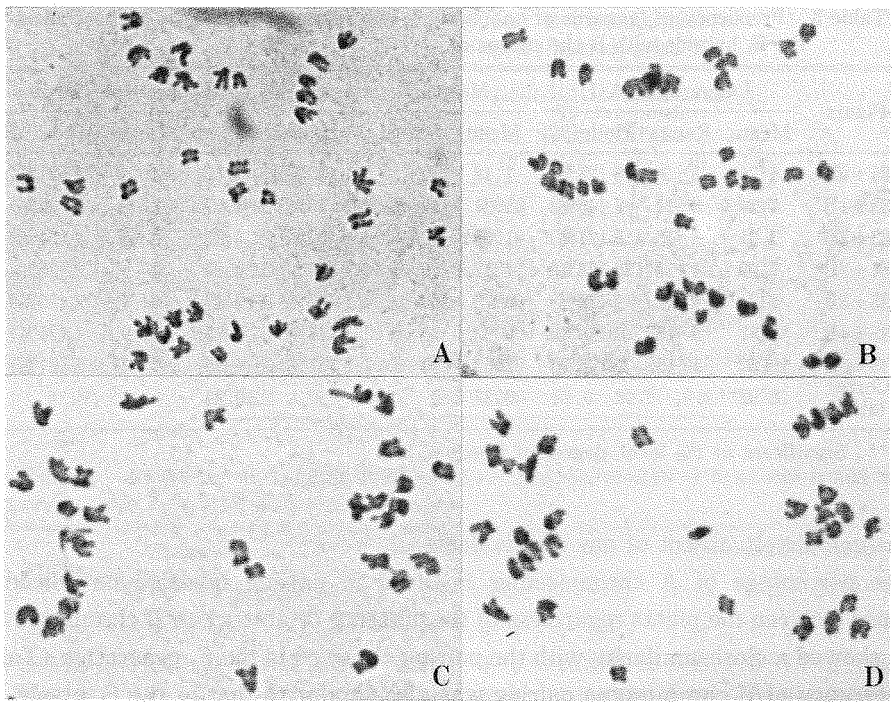

Fig. 3 Chromosome segregation at anaphase-1 in the F_1 (A, B) and F_2 (C, D) generations. A, 13-12-12 distribution in 35+2B plant. B, 13-15-12 distribution in 35+5B plant. C, 13-5-12 distribution in 29+1B plant. D, 15-4-14 distribution in 30+3B plant.

Table 2 Frequency of A and B chromosomes pairing at M-I in the F_1 hybrids with B chromosomes.

Plants	A chromosome pairing				B chromosome pairing		
	I	II	III	IV	I	II	III
35+0B	22.4	76.9	0.7	0.0	—	—	—
35+1B	20.3	72.7	6.8	0.2	100.0	—	—
35+2B	23.1	76.5	0.3	0.0	53.0	47.0	—
34+2B	25.6	74.4	0.0	0.0	49.0	51.0	—
35+3B	19.9	73.3	6.5	0.2	48.0	50.0	2.0
35+4B	21.3	75.3	3.2	0.2	46.5	53.5	0.0
35+5B	25.1	70.6	4.3	0.0	46.8	52.4	0.6

analyzed cytologically at meiosis. Meiotic chromosome association at M-I in plants with 29, 30 and 31 A chromosomes plus 1, 2 or 3 B chromosomes and the control plants with only corresponding number of A chromosomes are presented in Table 4 and Fig. 2 (E-F). The average A univalent per cell in plants with B chromosomes was slightly higher than the control but showed no significant correlation. Similarly average bivalent and trivalent per cell also showed non-significant correlation with the number of B chromosomes. The modal configuration almost exhibited with 14 II plus univalent of the A chromosoms, mostly, corresponding to their extra chromosome number of the D genome. The average number of univalent and bivalent per cell of the B chromosome was similar to the F_1 plants

Table 3 Frequency of laggards at anaphase- I and micronuclei at dyad and tetrad in the F_1 hybrids with B chromosomes.

Plants	Laggards at A-I			Micronuclei at dyad			Micronuclei at Tetrad		
	Mean	Range	Mode(%)	Mean	Range	Mode(%)	Mean	Range	Mode(%)
35+0B	6.26	3-9	6 (28%)	1.18	0-5	1 (35%)	4.43	2-10	4 (21%)
35+1B	7.14	3-11	7 (46%)	1.31	0-4	1 (39%)	4.97	2-11	5 (22%)
35+2B	7.31	3-13	8 (27%)	1.63	0-6	1 (33%)	3.33	0-8	4 (24%)
34+2B	7.99	4-14	8 (22%)	1.19	0-5	1 (40%)	2.30	0-6	2 (26%)
35+3B	8.24	4-12	9 (24%)	0.75	0-4	1 (37%)	2.47	0-7	2 (25%)
35+4B	8.87	5-15	10(19%)	0.93	0-5	1 (47%)	3.04	0-9	4 (26%)
35+5B	10.67	5-15	12(24%)	2.02	0-7	3 (31%)	4.00	0-10	5 (21%)
Correlation	0.92**			0.27			-0.24		

** : Significant at 1% level, degree of freedom, 5.

but was completely absent of any multivalents.

The percentage of A chromosomes involved in pairing revealed no considerable difference between the plants regardless of the presence or absence of B chromosomes and almost showed a close similarity with the pairing observed in the F_1 generation (Table 5). The frequency of B chromosome pairing was also similar to that of the F_1 plants.

The average frequency of laggards at A-I was significantly and positively correlated with the number of B chromosomes in F_2 plants (Table 6, Fig. 3 C-D). The frequencies of micronuclei both at dyad and tetrad were found to be increased due to B chromosomes in each category of plants with similar A chromosome number but correlation coefficients

Table 4 chromosome association at M-I in the F_2 hybrids with B chromosomes.

Plants	A chromosome association					B chromosome association				Number of cells
	Average				Mode	Average			Mode	
	I	II	III	IV		I	II	III		
29+0B	1.20 (0-3)	13.87 (9-14)	0.02 (0-1)	0.0 —	1 I+14 II	—	—	—	—	180
29+1B	2.63 (1-5)	13.11 (8-14)	0.05 (0-1)	0.0 —	3 I+13 II	1.0	—	—	1 I	144
29+2B	1.22 (0-4)	13.28 (7-14)	0.39 (0-2)	0.01 (0-1)	1 I+14 II	0.96 (0-1)	0.52 (0-1)	—	1 II	343
30+0B	1.53 (0-5)	13.86 (9-14)	0.25 (0-2)	0.0 —	2 I+14 II	—	—	—	(—)	126
30+2B	1.86 (0-3)	13.77 (10-14)	0.20 (0-2)	0.0 —	2 I+14 II	0.86 (0-2)	0.57 (0-1)	—	1 II	119
30+3B	2.32 (1-6)	13.82 (8-14)	0.01 (0-1)	0.0 —	2 I+14 II	1.78 (1-3)	0.61 (0-1)	0.0 —	1 I+1 II	223
31+2B	3.08 (3-5)	13.90 (9-14)	0.04 (0-1)	0.0 —	3 I+14 II	1.22 (0-2)	0.39 (0-1)	—	2 I	128
Correlation[1]	0.60	-0.14	-0.13	-0.08						

* : Figures in parentheses indicate the range.

[1] : Degree of freedom, 5.

Table 5 Frequency of A and B chromosomes pairing at M-I in the F_2 hybrids with B chromosomes.

Plants	A chromosome pairing				B chromosome pairing		
	I	II	III	IV	I	II	III
29+0B	4.1	95.7	0.2	0.0	—	—	—
29+1B	9.1	90.4	0.5	0.0	100.0	—	—
29+2B	4.2	91.6	4.0	0.1	48.0	52.0	—
30+0B	5.1	92.4	2.4	—	—	—	—
30+2B	6.2	91.8	2.0	0.0	43.0	57.0	—
30+3B	7.7	92.1	0.1	0.0	59.3	40.7	0.0
31+2B	9.9	89.7	0.4	0.0	61.0	39.0	0.0

Table 6 Frequency of laggards at anaphase-I and micronuclei at dyad and tetrad in the F_2 hybrids with B chromosomes.

Plants	Laggards at A-I			Micronuclei at dyad			Micronuclei at tetrad		
	Mean	Range	Mode(%)	Mean	Range	Mode(%)	Mean	Range	Mode(%)
29+0B	1.32	0-5	1 (59%)	1.22	0-4	1 (39%)	1.46	0-5	2 (38%)
29+1B	1.47	1-4	2 (50%)	2.47	0-6	2 (35%)	2.92	0-7	2 (27%)
29+2B	3.58	0-8	4 (46%)	2.84	0-4	2 (36%)	2.97	0-5	2 (41%)
30+0B	2.18	0-4	2 (32%)	1.01	0-2	1 (39%)	1.10	0-3	1 (44%)
30+2B	2.46	0-4	2 (36%)	1.17	0-2	1 (83%)	1.48	0-4	1 (38%)
30+3B	2.88	0-7	3 (48%)	1.35	0-4	1 (36%)	1.70	0-3	1 (52%)
31+2B	4.26	3-7	4 (62%)	2.31	0-7	3 (30%)	1.62	0-4	2 (32%)
Correlation	0.78*			0.11			0.10		

* : Significant at 5% level, degree of freedom, 5.

were nonsignificant (Table 6). The range and the modal number were comparatively higher in plants with Bs than the control plants.

Discussion

Assessment of the degree of relationship among species in the subtribe *Triticinae* has been based largely on the amount of pairing at meiosis in F_1 interspecific hybrids. There is a general agreement that the A and B genomes of the Emmer wheat are closely related to the A and B genomes of the Dinkel wheat, because most hybrids between Emmer and Dinkel wheats regularly formed fourteen bivalents at meiosis. However, in some hybrids fewer than fourteen pairs are formed. Since these chromosomes usually pair and disjoin normally, each of the gametes receives fourteen of these chromosomes. The seven univalent chromosomes of the D genome are, however, distributed at random to the gametes. Thus gametes produced by the F_1 have 14 chromosomes of the A and B genomes and 0 to 7 chromosomes of the D genome.

The result in the present investigation showed quite a similarity with the above expectation although the materials were not only the hybrids between tetraploid and

hexaploid wheat species, but also contained many B chromosomes of rye. The B chromosomes in the present hybrids could not substantially influence the meiotic pairing of the A chromosomes in terms of homoeologous as well as homologous pairing, since correlation coefficient between the number of B chromosomes and different associations of the A chromosomes were nonsignificant. In some cases homoeologous trivalent and tetravalent were observed between the chromosomes of D genome and the chromosomes of A or B genomes but no preponderance either in control plants without Bs or in plants with B chromosomes were noticed.

It is a well established fact that the number five chromosome of the B genome (5B) of wheat possesses a major regulatory gene or gene complex called pairing homoeologous (Ph) located in the long arm, which regulates homoeologous pairing between the chromosomes of different genomes[3, 4]. In species which may have contributed the 5B chromosome of wheat or which may have related to it, the same allele is not encountered, although several other genes affecting homoeologous pairing are present. The supernumerary B chromosomes (not 5B chromosome of B genome) of at least two such species e.g. *Aegilops speltoides* and *Ae. mutica* however, have been found to have an effect very similar to that of Ph allele of wheat[9, 10, 14]. In *Lolium* species hybrid, accessory B chromosomes have been found to suppress homoeologous chromosome pairing [15, 16, 17, 18]. However, Roothaan and Sybenga[19] stated that the B chromosomes of rye were entirely ineffective in promoting or suppressing homoeologous pairing in hybrids of wheat × rye with B chromosomes. But Hossain *et al.*[20] reported that in the hexaploid *Triticale* with many B chromosomes of rye, the latter substantially decreased pairing between the homologous chromosomes. In the present pentaploid (F_1) and hyper-tetraploid wheat (F_2), in the presence of 1 to 7 chromosomes of the D genome of wheat, homologous as well as homoeologous pairing between the autosomes were not influenced irrespective of the presence or absence of rye B chromosomes. While, in hexaploid wheat rye B chromosomes had a negative influence on the homologous pairing between the A chromosomes[21]. Therefore, in the absence of pairing partners of the chromosomes of the D genome, the univalent chromosomes of the above genome may have some sort of buffering effect on the B chromosomes activity, while such buffering ability was neutralized when they paired in hexaploid wheat.

Comparatively, the much higher frequency of laggards at anaphase-I observed in both the F_1 and F_2 plants might be due to less pairing between the B chromosomes at M-I, since the correlations between the number of Bs and A chromosome univalent were nonsignificant. However, it is also very difficult to identify clearly the B chromosomes from A compliment at anaphase-I. Contrary to the high frequency of laggards, micronuclei at dyad and tetrad were lower than expected, being indicative of that a majority of the lagging chromosomes, especially the Bs, might have incorporated into the daughter nuclei. The degree of elimination of the B chromosomes was very little, as judged from the transmission of B chromosomes in the offspring, while the univalents of A chromosome were rapidly lost from the hybrids.

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ライムギB染色体を有する普通系コムギ（*Triticum aestivum*）と二粒系コムギ（*Triticum durum*）の交雑によるF_1，F_2の細胞学的研究

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摘　　要

数本のライムギB染色体を有する普通系コムギ（*Triticum aestivum*）と二粒系コムギ（*Triticum durum*）の交雑によるF_1，F_2の細胞学的研究を行なった。F_1，F_2で，Dゲノムは，ほとんど一価染色体として存在するが、AゲノムとBゲノムは二価染色体と稀に三価染色体を形成した。F_1のコムギ五倍体およびF_2の低五倍体の染色体対合について，ライムギB染色体の影響はなかった。F_1，F_2世代で，B染色体間の対合の親和性の低下により第1分裂後期の遅滞染色体数の増加はB染色体数と正の関係が認められた。この交雑では，一価のA染色体は急激に失われたがB染色体はさほど減少しなかった。